Significant β -carboline Alkaloid Harmine in Different Developmental States of Mammals in Vivo: Endogenic or Exogenic?

Ning Cao

Shanghai University of Traditional Chinese Medicine

Shuping Li

Shanghai University of Traditional Chinese Medicine

Aimin Xu

Kashi Prefecture First People's Hospital

Xiaoguang Zou

Kashi prefecture First People's Hospital

Zunji Ke

Shanghai University of Traditional Chinese Medicine

Gang Deng

Shanghai University of Traditional Chinese Medicine

Xuemei Cheng

Shanghai University of Traditional Chinese Medicine

Chang hong Wang (✉ wchcxm@hotmail.com)

Shanghai University of Traditional Chinese Medicine <https://orcid.org/0000-0001-7578-4856>

Research article

Keywords: Harmine, β -Carboline alkaloids, Exposure levels, Endogenous substance

Posted Date: August 18th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-806480/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Several β -carboline alkaloids (β CBs), harmine, harmaline, harmane, and nor-harmane, are effective for Alzheimer's disease (AD) model mice. They were discovered in some plants, common foodstuffs, and in blank plasma of various mammals. However, whether these compounds in mammals are exogenous or endogenous remained unclear. The exposure levels of these β CBs in plasma and tissues of pup rats, aging rats and volunteer were detected by UPLC-MS/MS.

Result: Results showed that harmine was the main compound found in rats, mice, human, and even in newborn rats without consumption. The changes of harmine concentration showed a high dependence with growth (aging), gender and race. And the concentration of harmine in rat plasma showed a decreasing trend during the growth period of 18 months.

Conclusion: These results revealed that harmine may be relevant to AD. The exposure level of harmine in plasma indicate that in addition to exogenous ingestion, spontaneous synthesis might be another important source of harmine in mammals.

Introduction

β -carboline alkaloids (β CBs), harmine, harmaline, harmane, and nor-harmane, are active components of *Peganum harmala* [1]. *P. harmala* is traditionally used to treat diseases, such as cough, asthma, rheumatoid arthritis, and swelling pain in the Middle East, central Asia, and South America [2]. In addition, such β CBs show various pharmacological effects, such as hypoglycemic effect, antineoplastic activity, and anti-acetylcholinesterase (AChE) activity [1]. These β CBs are also present in other plants, including *Banisteriopsis caapi*, *Tribulus terrestris*, and *Ayahuasca* [3]. Such β CBs can also be found in common foodstuffs, such as plant-derived foods (e.g., grapes, rice, corn, barely, bean, and rye), processed foods (e.g., wine, beer, whisky, brandy, sake, coffee, vinegar, and tobacco), and meat products (e.g., barbecue, smoked fish, and smoked sausages) [4]. Moreover, harmane and nor-harmane are widely distributed in the brain, liver, blood, and urine of human and many other mammals [5–7]. Louis et al. reported that the plasma contents of harmane in essential tremor patients and Parkinson patients were higher than those in healthy individuals [6, 7]. With the deepening of research, harmine, harmaline, and other β CBs have been found in blank plasma of adult rats and mice without any consumption [3]. Consequently, whether harmine, harmaline, harmane, and nor-harmane are endogenous or exogenous in mammalian tissues and plasma and whether such β CBs in mammals are useless or functional are still unclear.

β CBs are synthesized by the Pictet–Spengler reaction (P–S reaction) in plants [8]. Strictosidine synthase is a vital enzyme in the reaction [9]. In addition, a protein similar to strictosidine synthase is found in the human brain, which may indicate that the P–S reaction may also occur in human being [10]. Tryptamine is a synthetic precursor of the P–S reaction. Moreover, tryptamine is widely distributed in the brain [11]. Thus, hypothesis has been carried out that harmaline, harmine, harmane and nor-harmane may be self-synthesized in mammals. Moreover, the function of such β CBs in mammals is still unknown.

Based on previous reports, β CBs can play various pharmacological effects, such as anticoagulant activity, hypoglycemic effect, antineoplastic activity, antioxidative, and anti-inflammatory activity [1]. These β CBs are either AChE and butyrylcholinesterase inhibitors or monoamine oxidase A inhibitors [1]. In addition, the abovementioned four alkaloids can bind with opioid receptors, imidazoline I_2 receptors, and 5-hydroxytryptamine (5-HT) receptors, which contribute to the analgesic effect, reduction of withdrawal syndrome, and regulation of neurotransmitters [1]. Moreover, neurotoxicity of harmine, harmaline, harmane, and nor-harmane is an indivisible side effect, which leads to essential tremor [12].

Based on our published research and results, harmine, harmaline, harmane, and nor-harmane are effective in mice with nervous system disease, such as Alzheimer's disease (AD) [13]. AD is a common neurodegenerative disease and characterized by a decline in memory, language, and other cognitive skills [14]. Although no conclusion has been found on the pathogenesis of AD, several recognized hypotheses have been carried out. One popular mechanism is self-replication and spreading of A β and Tau aggregates [15]. A β deposition in transgenic mice, not labeled by thioflavin or Congo red-based probes, is similar to AD patient brain tissue [16]. In addition, brain's cholinergic system plays an important role in AD. Cholinesterase inhibitors can increase the availability of acetylcholine at synapses in the brain [16]. The effective cholinesterase inhibitors such as donepezil, galantamine, and rivastigmine have been proven clinically useful in AD treatment [17]. Moreover, neurotransmitters play an indispensable role in AD development, and aberrant neurotransmitter release at synapses can cause cognitive decline in AD [18]. Researchers have found that AD is associated with inadequate levels of various neurotransmitters [15]. The AChE inhibitory activity and the regulation of neurotransmitters are possible underlying mechanisms of harmine, harmaline, harmane, and nor-harmane in the treatment of AD model mice. Thus, such alkaloids, which are widely distributed in mammals' body fluids, may be beneficial to the prevention and treatment of AD.

Therefore, harmaline, harmine, harmane, and nor-harmane may be endogenous substances, and a potential connection can be found between those endogenous alkaloids and AD.

The plasma of rats and human at different ages is tested, particularly newborn pup rats, to verify whether harmaline, harmine, harmane, and nor-harmane are endogenous and naturally present in mammal body. AD is a cognitive impairment disease, which is closely connected with ages. Brain development in childhood is crucial to cognitive function [19]. Memory and learning ability have developed from infancy to adulthood according to processing speed, working memory, language, and visuospatial test [19]. However, cognition decreases with aging [20]. According to research published in 2016, most people with AD are age 65 or older, and 15% of those with AD are ages 65–74, whereas 44% are ages 75–84 [14]. Growth and aging are important processes of cognitive level changes. Thus, the contents of harmine, harmaline, harmane, and nor-harmane in different developmental states of mammals are determined.

Plasma of AD model mice is tested and compared with control animals according to the possible mechanism of AD to confirm whether the content variation of harmine, harmaline, harmane, and nor-harmane in mammal plasma implies the occurrence of AD.

In general, the abovementioned research is important to determine the origin and function of harmine, harmaline, harmane, and nor-harmane in mammal plasma. Studies on the connection among plasma contents of such β CBs in different developmental states of mammals can deepen studies on the endogenous physiological effects of such alkaloids.

Results

Alkaloid exposure levels in the developmental phase of newborn rats within 29 days after birth

The validated analytical method was applied to study the exposure levels of alkaloids in plasma and various tissues with the growth of pup rats within a month. The plasma concentrations of harmine were calculated by using the calibration curve. However, the concentrations of harmaline, harmane, and nor-harmane were difficult to count when the contents were below the lower limit of detection. As shown in Fig. 2, the contents of harmine in plasma and other organs showed different variations. First, harmine was detected in plasma and all of the tissues. The contents were listed as follows: plasma: 0.16 ± 0.03 ng/mL; brain: 0.33 ± 0.14 ng/g; heart: 0.34 ± 0.15 ng/g; liver: 0.26 ± 0.11 ng/g; spleen: 0.37 ± 0.12 ng/g; lungs: 0.46 ± 0.11 ng/g; kidney: 0.44 ± 0.13 ng/g; genital organ: 0.39 ± 0.12 ng/g; white fat: 0.33 ± 0.07 ng/g; brown fat: 0.36 ± 0.17 ng/g; muscle: 0.31 ± 0.18 ng/g. The concentrations of harmine exerted markedly different variation with the passage of time because of the different developmental characteristics of different organs. The concentrations of harmine in the plasma, brain, white fat, and genital organ were trending downward, whereas other organs (heart, liver, spleen, lungs, kidney, muscle, and brown fat) showed no significant trend with the growth of pup rats. The contents in plasma tended to be stable on the ninth day, but on the fifteenth day, the concentrations in the brain became stable. The contents in the genital organ and white fat did not appear plateau. The brain was a crucial functional organ of harmine, whereas the liver was a vital metabolic organ. Then, the amounts of harmine in various tissues were calculated, and the amount–time curves were drawn (supplementary materials Fig. S2). The amount of harmine increased in tissues of the brain, heart, liver, spleen, lungs, and kidney with the growth of pup rats. In tissues of the heart, lungs, and kidney, the amounts of harmine increased with the development of organs until the third week after birth. Notably, the amounts of harmine in the brain increased faster than the development of body weight in the second week after birth.

Harmine, harmaline, harmane, and nor-harmane were not detected in fodder and bedding. A weak signal response was detected at the same peak time in harmine, and all of the responses were below the lower limit of quantification after the supernatant was concentrated 50 times. The detail dates are shown in supplementary materials Table S5.

Exposure levels of alkaloids and neurotransmitters in the growing process of 18-month-old rats (from youth to old stage)

The alkaloids in plasma with the aging of rats were determined by a validated analytical method. As shown in Fig. 3, the concentrations of harmine in plasma decreased gradually with aging. The contents of harmine reached 1.80 ± 1.51 ng/mL in the first month and reduced to 0.35 ± 0.04 ng/mL in the

sixteenth month. The concentrations tended to be stable in the sixth month and maintain at a low concentration.

Meanwhile, neurotransmitter concentration in plasma with the aging of rats was determined by a validated analytical method. As shown in Fig. 4, various neurotransmitters showed different trends (concentration range, mean $\pm$ SD): 5-HIAA (154.7–691.0 ng/mL, 384.3 ± 151.3 ng/mL), 5-HT (45.4–9850.2 ng/mL, 2653.0 ± 2644.0 ng/mL), ACh (30.1–137.7 ng/mL, 63.4 ± 25.4 ng/mL), Ch (112.1–1526.1 ng/mL, 613.0 ± 295.1 ng/mL), Glu (778.2–5998.6 ng/mL, 1878.9 ± 914.8 ng/mL), L-Trp (11006.5–44853.4 ng/mL, 21091.9 ± 5915.5 ng/mL), Phe (5734.4–16919.3 ng/mL, 9169.1 ± 1703.7 ng/mL), and Tyr (10098.0–32219.6 ng/mL, 20339.2 ± 4809.8 ng/mL). The exposure levels of 5-HT, ACh, Glu, L-trp, and Phe reduced with aging, but the contents of 5-HIAA, Ch, and Tyr showed no significant tendency over time.

Exposure levels of alkaloids in different physiological states of mice

As shown in Fig. 5, in different physiological states of mice, the contents of harmine, harmane, and harmaline were different. As shown in Fig. 5A, the contents of harmine and harmane in wild-type mice plasma (harmine: 0.43 ± 0.26 ng/mL, harmane: 0.095 ± 0.078 ng/mL) were higher than those in APP/PS1 double transgenic mice (harmine: 0.28 ± 0.060 ng/mL, harmane: 0.085 ± 0.028 ng/mL). However, the concentrations of harmaline in wide-type mice plasmas (0.20 ± 0.22 ng/mL) were lower than those APP/PS1 double transgenic mice (0.26 ± 0.38 ng/mL). As shown in Fig. 5B, the contents of harmine and harmane in normal mice plasma (harmine: 0.60 ± 0.28 ng/mL, harmane: 0.23 ± 0.15 ng/mL) were higher than those in scopolamine-mode mice (harmine: 0.54 ± 0.37 ng/mL, harmane: 0.17 ± 0.11 ng/mL). By contrast, harmaline showed opposite results (control: 0.11 ± 0.034 ng/mL, model: 0.15 ± 0.080 ng/mL).

Exposure levels of alkaloids in different developmental and physiological states in human

In human plasmas, the concentrations of nor-harmane were below the lower limit of detection or could not be determined in most samples, and the results of the other three alkaloids are summarized in Fig. 6. The contents of harmine were higher than harmane and harmaline in plasmas. The participants were divided into groups according to ages, gender, ethnicity, and living habit. Harmine (1.90 ± 3.27 ng/mL) and harmaline (0.36 ± 0.90 ng/mL) concentrations in individuals below 60 years old were significantly higher than those above 60 years old (harmine: 0.99 ± 0.54 ng/mL; harmaline: 0.13 ± 0.07 ng/mL; Fig. 6A, $P < 0.001$). The plasma contents of harmine and harmaline in female and male were significantly different, and the contents in female were higher (Fig. 6B, $P < 0.05$). Detail information were shown as follows: harmine (female: 2.03 ± 3.07 ng/mL; male: 1.57 ± 2.16 ng/mL) and harmaline (female: 0.57 ± 1.32 ng/mL; male: 0.24 ± 0.53 ng/mL). In plasma samples from participants with different ethnicities, the plasma concentrations of harmine and harmaline of Han nationality (harmine: 1.82 ± 2.74 ng/mL; harmaline: 0.42 ± 1.00 ng/mL) were higher than that of Uighur (harmine: 1.47 ± 1.72 ng/mL; harmaline: 0.17 ± 0.38 ng/mL), which showed significant difference (Fig. 6C, $P < 0.05$). Drinking was a factor affecting the contents of harmaline, and the harmaline level of individuals who did not drink (0.42 ± 1.02

ng/mL) were higher than those who drunk (0.22 ± 0.57 ng/mL; Fig. 6D, $P < 0.05$). However, harmine was not affected ($P > 0.05$). Smoking did not affect the concentration of the three compounds in plasmas (Fig. 6E, $P > 0.05$). The concentrations of harmine showed no difference among different ages, genders, and ethnicities ($P > 0.05$).

Discussion

The content of harmine, harmaline, harmine, and nor-harmine in different developmental and physiological states of rat was detected to explore the origin and function of such alkaloids. Limited by the detecting condition, the concentrations of harmaline and harmine in some rat plasma samples were below the LLOQ. As for nor-harmine, it was not determined in many samples; thus, nor-harmine had no results. Harmine was detected in newborn pup rat plasma and tissues. Harmine was discovered in each tissue of pup rats, including the brain, heart, liver, spleen, lungs, kidney, muscle, white fat, brown fat, and genital organ. In addition, researchers found harmine in pig and rat brain [3]. The exposure level of harmine in rat plasma showed a decreasing trend with aging. Notably, harmine was found in the whole life of rat. In human plasma, harmine showed differences with regard to age, gender, race, and physiological and pathological status. Therefore, harmine is a naturally and widely distributed endogenous substance in mammals. The concentrations of harmaline and harmine were lower than harmine in most plasma of rats, mice, and human. Harmaline could turn to harmine in vivo with the presence of heme peroxidase [21], which might account for the contents of harmine were higher than harmaline under normal conditions. Based on the aforementioned research, harmine was more likely to be endogenous compounds than the other three alkaloids because harmine was detected in each rat, mouse, and human plasma, whereas the other three compounds were not.

Determining the contents of the abovementioned alkaloids in pup rat plasmas could help to distinguish alkaloids in blank plasma whether from food intake. Notably, harmine could be detected in plasma and other tissues of newborn rats without consuming any foodstuffs containing target alkaloids. Meanwhile, the fodder and bedding were detected free of harmine, and the exogenous disturbances from the growth environment of rats could be further excluded. Despite a weak signal response at the same peak time in harmine, the response was below the lower limit of detection. Thus, the trace amount of harmine, harmaline, harmine, and nor-harmine in fodder and bedding could be ignored. On the contrary, if the concentration of harmine was the lower limit of detection, then the concentrations of harmine in fodder would reach 0.012 ng/g. The maximum daily food consumption of SD rats was approximately 20 g, and the bioavailability of harmine was 17% [3, 12]. Considering that the blood volume accounted for 8% of the body weight of rats, combined with the daily intake of 20 g of each rat and the bioavailability of about 17% of harmine, the exogenous intake of harmine would only account for 1.3% of the total blood drug concentration. Therefore, based on the results of this experiment, harmine in newborn rat plasma and other tissues is natural rather than exogenous uptake.

Based on previous research of the synthesis of β CBs in plants, the P-S reaction was an essential part in the whole synthetic route, in which the strictosidine synthase was the vital enzyme in life [9, 22]. The P-S

reaction easily occurred without enzyme catalysis in food cooking, including roasted coffee, barbecue, and toasted bread [4]. The structure and function of strictosidine synthase in plants have been determined [23]. Except for tryptamine and secologanin, other amines and aldehydes could be a substrate according to the substrate specificity studies [23]. Furthermore, protein similar to strictosidine synthase was found in other life forms, including human [8]. In addition, tryptamine, one of the substrates, is a popular compound in mammals [11]. Therefore, harmine may be synthesized in vivo in mammals by using tryptamine as one of the substrates under catalysis by synthesizing proteins similar to strictosidine in plants. The supplementation of precursors, such as tryptamine and other amine with secologanin, and other aldehyde, may be greater than the direct addition of harmine. However, more experiments are needed to confirm the underlying mechanism.

Based on previous reports, harmine could stimulate proliferation of human neural progenitor cells and inhibit the dual specificity tyrosine-phosphorylation-regulated kinase, which regulated brain development [24]. Harmane could activate the firing and burst activity of dopamine neurons, and the increase in firing rate produced by harmane was greater than that produced by nicotine [25], which may imply that harmine has a similar effect because of similar structure. In addition, tryptamine, the synthetic precursor of harmine, was widely distributed in the brain [11]. Therefore, harmine could play a role in the development of the nervous system. The sharp reduction of harmine in developmental state may indicate that harmine primarily functions in the early stage of nervous system growth and development.

The developmental and physiological states of newborn rats experienced great changes in a month, and pup rats from different maternal rats usually had inborn differences [26]. Thus, sampling time and grouping are important factors. The dates of each sampling point were obtained from ten different maternal rats to avoid inborn differences and make the experiment more accurate. The sampling times were different because they were not delivered in the same day. Thus, the experiment eliminated congenital difference of pup rats and reflected the variation trend of harmine in various tissues and plasma with the development of pup rats.

AD was a deadly progressive neurodegenerative disorder, and the pathogenesis of the disease had no clear conclusion at present [16]. The brain exhibited signs of compromised bioenergetics with aging, including inflammation, accrual of oxidatively modified molecules, and other impairments. In addition, aging individuals were vulnerable to AD or other neurodegenerative diseases [27]. Researchers directly associated the disease with aging but not specifically with the theories of aging in general. Distinguishing normal aging from AD is difficult because aging is a main risk factor for acquiring AD [28]. Researchers showed the relationship between aging and AD, and they regarded the aging rodent models as an AD model [28]. Many factors of aging could alleviate AD phenotypes, and drugs and treatment for AD could slow the aging phenotypes [29]. Harmine was a compound that enhanced the spatial cognition of C57BL/6 mice and regulated the concentration of various neurotransmitters [13]. Thus, administration of harmine could improve spatial cognition in aging rats. On the contrary, the lack of harmine might result in the development of AD during aging.

The contents of 5-HT, ACh, Glu, γ -Trp, and Phe in rats reduced with aging. In addition, the contents of such neurotransmitters were lower in plasma of AD model mice with intraperitoneal injection of scopolamine than those of normal mice [13]. AD was associated with inadequate levels of a variety of neurotransmitters [15]. Neurotransmitters played a significant part in brain circuit involved in many aspects of learning and memory, particularly the serotonergic, glutamatergic, and cholinergic neurotransmitters [15]. This result indicated the connection between aging and AD.

The AD model mice were determined to clarify the connection between AD and alkaloids. Scopolamine was a nonselective antagonist of the muscarinic cholinergic receptor, which led to cognitive deficits associated with the reduction of cholinergic neurotransmission [18]. APP/PS1 double transgenic mice showed typical A β pathology and memory impairment in an age-dependent manner [30]. The APP/PS1 double transgenic mice presented similar exposure rule to the scopolamine model mice compared with control. The contents of harmine, harmaline, and harmane in those two model mice showed no significant difference compared with the control. Although harmine, harmaline, and harmane could enhance the cognitive ability of AD model mice, the occurrence of disease played little role in the regulation of these alkaloids.

Harmaline and harmine showed a significant difference among young and old people because old people could be easily attacked by AD. The reduction of harmine and harmaline may account for the easy attack because of efficient pharmacology in AD [1]. In addition, when the neuronal cell of the central nervous system was senescent, aged neurons showed signs of impaired cellular signaling, which exhibited memory decline [31]. The results were consistent with the reduction of harmine in aging rat plasma.

Based on the results, the contents of harmaline and harmine in female plasma were higher than those of male plasma ($P < 0.05$). The result may be caused by the different expression of cytochrome enzyme in women and men. Harmaline and harmine were metabolized by CYP2D6 [3]. The expression of CYP2D6 in female was lower than that of male according to research, and the response to opioids showed gender differences [32, 33]. The difference in the expression of CYP2D6 accounted for the significant difference in the plasma contents of harmine and harmaline in female and male. On the contrary, no gender difference was associated with CYP1A2 activity [32]. Thus, no gender difference was found on the plasma concentrations of harmane.

The concentrations of harmaline and harmine in plasma of Han people were higher than those of Uyghur ($P < 0.05$). CYP2D6 was the main metabolic enzyme of harmaline and harmine [34]. Reduced functional allele CYP2D6*10 was important in Asian population, and Asians had high frequency of reduced functional allele (median = 41%) [35]. Then, CYP2D6*10 contributed to the population shift to metabolic rates, indicating slower metabolism [36]. Researchers compared the expression of CYP2D6*10 in the Han and Uyghur blood. The result showed that the expression of CYP2D6*10 in Han was higher than that in Uyghur [37]. Consequently, the metabolic rate of CYP2D6 in Uyghur was faster than that in Han, and the concentrations of harmaline and harmine in Han plasma were higher than those of Uyghur. Different from harmine and harmaline, CYP1A2 was the main metabolic enzyme of harmane [38]. Moreover, no

significant difference of CYP1A2 was found between Han and the Uyghur. Therefore, the concentrations of harmane showed no difference between the Han and Uyghur plasma.

No significant differences in such alkaloids were found among smokers and drinkers, except for harmaline. The plasma concentration of harmaline in drinkers was lower than that of nondrinker ($P < 0.05$). This finding was consistent with various reports that ethanol consumption could produce an adverse impact on AD and increase the risk of AD development [39]. Increasing drinks of hard liquor could faster the rate of cognitive decline while mild-moderate drinks were not [40]. Such studies showed the correlation between drinking and disease, but no finding indicated a causal impact of alcohol on AD. Thus, the degree of drinking and standardization of alcohol may be a vital element for further research.

Harmine in mammals was obtained from endogenous synthesis rather than exogenous uptake. In addition, the behavior of the abovementioned three alkaloids in human, rats, and mice plasmas was consistent with the regulation of AD development. Furthermore, the abovementioned results showed the surface phenomenon of the alkaloid exposure regulation in several mammals. The endogenous characteristic would be verified only if the synthetic pathway in vivo was found.

Conclusions

Harmine is a widespread β CBs in mammals, and it could be discovered in the plasma of rat, mouse, and human. Harmine was found in newborn rat plasma and many tissues without any consumption. In addition, changes of harmine concentration showed a high dependence with growth (aging), gender and race. With the increase of age, the concentration of harmine in rat plasma showed a decreasing trend. These results might imply that harmine is associated with AD. However, further studies were warranted to determine the underlying mechanism of endogenous synthesis and the specific function of harmine in mammals. This study was the first to investigate the variation of harmine concentration in mammals in different developmental status. The exposure level of harmine in plasma indicate that in addition to exogenous ingestion, spontaneous synthesis might be another important source of harmine in mammals.

Materials And Methods

Reagents and Materials

Harmine, harmane, and harmaline (purity > 98%) were isolated by HPLC from seeds of *P. harmala* in our laboratory. Nor-harmane was purchased from Sigma Aldrich Co. (St. Louis, MO, United States). Scopolamine hydrobromide was purchased from TCI (Shanghai) Development, Co., Ltd. (Shanghai, China). L-tryptophan (L-Trp), 5-hydroxytryptamine (5-HT), 5-hydroxyindole-3-acetic acid (5-HIAA), acetylcholine chloride (ACh), choline chloride (Ch), L-glutamic acid monosodium salt monohydrate (L-Glu), L-phenylalanine (L-Phe), L-tyrosine (L-Tyr), theophylline, tacrine (internal standard), and heparin sodium were purchased from Sigma Aldrich Co. (St. Louis, MO, United States). Perchloric acid and sodium hydroxide were purchased from Meilunbio® Biotech, Co. Ltd. (Dalian, China). Bovine serum albumin was

purchased from YEASEN Biotechnology, Co. Ltd. (Shanghai, China). Acetonitrile, methanol, and formic acid of HPLC grade were purchased from Fisher Scientific, Co. (Santa Clara, CA, United States). Deionized water (> 18 mΩ) was purified by Milli-Q Academic System (Millipore, Corp, Billerica, MA, United States).

Animals

Twenty adult and 11 pregnant Sprague-Dawley rats and 10 C75BL/6 mice were obtained from the Drug Safety Evaluation and Research Center of Shanghai University of Traditional Chinese Medicine. Ten APP/PS1 double transgenic mice in C57BL/6 background aged 5–6 months with their age-matched littermates were obtained from Nanjing Biomedical Research Institute of Nanjing University (Nanjing, China). Animals were introduced to the experimental holding rooms at least two days prior to commencement of the study. Animals were housed in a well-lighted air-conditioned room under standard environmental conditions (room temperature and relative humidity were kept at 25°C ± 1 °C and 60–65%, respectively) and given free access to rodent chow and tap water prior to the study. All animal-use procedures were in accordance with the regulations for animal experimentation issued by the State Committee of Science and Technology of the People's Republic of China on 14 November 1988 and the protocol was approved by the Animal Ethics Committee of Shanghai University of Traditional Chinese Medicine (NO. PZSHUTCM190912018; Approval date: 12 September, 2019).

Voluntary subjects

Participants were recruited from two cohorts: Shanghai University of Traditional Chinese Medicine (i.e., the SH cohort) and Kashi Prefecture First People's Hospital, Xinjiang China (i.e., the XJ cohort). All of participants were free from any neurodegeneration. A total of 131 participants were recruited from the SH cohort. Most of them were students aged from 24 to 30, and 15 volunteers were staffs aged from 31 to 55. The other 419 participants were enrolled from the XJ cohort. All of them went to Kashi Prefecture First People's Hospital for physical examination. The 419 healthy volunteers were in the age range of 19 to 81. All subjects were given informed consent and asked no intervention before and throughout the study period. Exclusion criteria were not available. The study was approved by the Research Ethics Committee of Kashi Prefecture First People's Hospital, and all participants provided informed consent (NO.2017-03; Approval date: February 28, 2017).

Analysis of target βCBs in plasma and tissues of mammals by UPLC-ESI-MS/MS

The concentrations of alkaloids were quantified with SHIMADZU LC-30AD UPLC system (Shimadzu, Kyoto, Japan) connected to an AB Sciex QTRAP® 6500 triple quadrupole mass spectrometer (SCIEX, United States) equipped with an ESI source using positive ion detection mode for multiple reaction monitoring. According to a previously validated method, chromatographic separation was conducted using a UPLC BEH C₁₈ column (50 mm × 2.1 mm, 1.7 μm, Waters, USA) [41]. Given the difference of linearity range, the sample pretreatment was slightly adjusted, and the methods and results are shown in supplementary materials Fig. S1 and Table S1–S3. The mobile phase consisted of an aqueous solution of 0.1% formic acid (solvent A) and an acetonitrile (solvent B) with a flow rate of 0.4 mL/min. The

gradient elution was established as follows: 0–2.5 min, 9–13% B; 2.51–3 min, 14–14.5% B; 3–4 min, 14.5–15.5% B; 4–5 min, 15.5–90% B; 5–6 min, 90–90% B; 6–7 min, 90–9% B; 7–8 min, 9% B. All other instrumental parameters were set according to previous studies, and the method was well validated and successfully applied to determine the concentrations of alkaloids [41].

Analysis of neurotransmitters in plasma of mammals by UPLC-ESI-MS/MS

The concentrations of neurotransmitters were determined with SHIMADZU LC-30AD UPLC system (Shimadzu, Kyoto, Japan) connected to an AB Sciex QTRAP® 6500 triple quadrupole mass spectrometer (SCIEX, US) equipped with an ESI source. Chromatographic separation was conducted using a ZIC-cHILIC column (150 mm × 2.1 mm, 3 µm) with a SeQuant ZIC-cHILIC guard column (20 mm × 2.1 mm, 5 µm, Merck-Sequant, Germany). The mobile phase was an acetonitrile (A)–water mixture containing 0.1% of formic acid (B), and the gradient elution was established as follows: 0–8 min, 65% A. All other instrumental parameters and sample pretreatment were set according to previous studies, and the method was well validated and successfully applied to determine the concentrations of neurotransmitters [42].

Exposure levels of target alkaloids in the developmental phase of newborn rats within 29 days

Eleven Sprague-Dawley pregnant rats were used in this study. The pregnant rats were raised under environmentally controlled room and with free access to food and water all throughout the experiment. After parturition, pup rats were grouped into 11 on the basis of mother rats. The individual group indicated one sampling point and contained 10 pup rats obtained from 10 different mother rats ($n = 10$). The pup rats were sacrificed at postnatal days 1 (P1), 3 (P3), 5 (P5), 7 (P7), 9 (P9), 12 (P12), 15 (P15), 18 (P18), 21 (P21), 25 (P25), and 29 (P29) for sample collection, in which 11 sampling points were collected. The specific time is shown in Fig. 1A. Each pregnant rat could give birth to around ten pup rats, and every pup rat was sacrificed at certain time to ensure each point in time contained 10 samples (half female and half male). The sex of pup rats was determined by anogenital distance. Pup rats were anesthetized at specified time for sample collection, and the grouping and sampling time is shown in supplementary materials Table S4. Prior to blood sampling, the pups were anesthetized to unconsciousness with 4% isoflurane via isoflurane vaporizers. After blood collection, pup rats were sacrificed by inhaling excessive isoflurane, and the tissues were collected. Approximately 0.5 mL of blood sample was collected from the angular vein of each rat and transferred into a 1.5 mL heparinized tube. The supernatant plasma (100 µL) was transferred into another 1.5 mL centrifuge tube after centrifugation of blood at $3000 \times g$ at 4 °C for 10 min. The tissues of the brain, heart, liver, spleen, lung, kidney, genital organ, muscle, white fat, and brown fat of each pup rat were also collected. Various tissues were weighted and stored at a suitable tube. All plasma and tissue samples were stored at – 80 °C until analysis.

Exposure levels of alkaloids in fodder and bedding

The concentrations of harmine, harmaline, harmane, and nor-harmane in the fodder and bedding were detected. The fodder and bedding were obtained from cages of above 11 pregnant rats and their pup rats.

Detection method was referred to quality specification established by Yang et al. [43]. The fodder and bedding were accurately powdered and weighted. The powders were added to methanol, which was 25 times its volume, and ultrasound treated for 25 min. In addition, power and frequency were kept at 250 W and 30 kHz, respectively. Afterward, supernatant (5 mL) was transferred to another tube and evaporated to dry at 37 °C under a slight stream of nitrogen. The dried residue was reconstituted with 100 µL of 9% acetonitrile and vortexed for 2 min. After centrifugation at $13000 \times g$ at 4 °C for 10 min, 20 µL of supernatant was injected into the UPLC-ESI-MS/MS system for alkaloid analysis.

Exposure levels of alkaloids and neurotransmitters in the growing process of 18-month-old rats (from youth to old stage)

Twenty Sprague-Dawley rats (200–220 g) with 10 males and 10 females were used in this study. The rats were raised under environmentally controlled room and with free access to food and water all throughout the experiment. The experimentation lasted for 16 months. The blood samples were collected once a month, and blood was drawn at around 9:00 am to 11:00 am. The schematic diagram is shown in Fig. 1B. Blood samples were collected from the angular vein for hematological analyses every month after rats were anesthetized to unconsciousness with 4% isoflurane via isoflurane vaporizers. The blood samples were promptly centrifuged at $3000 \times g$ at 4 °C for 10 min, and all of the supernatant plasma was transferred into a new 1.5 mL centrifuge tube. Plasmas were stored at – 80 °C until analysis. After sample collection, the plasmas were used for the analyses of some alkaloids and neurotransmitters after pretreatment.

Alkaloid exposure levels in different physiological states of mice

Two AD mouse models were used, including 10 male APP/PS1 double transgenic mice and 10 male scopolamine molding mice. All mice were housed under environmentally controlled room and with free access to food and water before experiment. The APP/PS1 double transgenic mice were proven to have impaired spatial learning and memory compared with C57BL/6 through the Morris Water Maze test [44]. Mice were anesthetized to unconsciousness with 4% isoflurane, and the blood were collected from the right orbital vein [44]. The blood samples were centrifuged at $3000 \times g$ at 4 °C for 10 min. Then, the supernatant plasmas were collected and stored at – 80 °C until analysis. Another AD mouse model was male C57BL/6 mice molded through intraperitoneal injection of scopolamine (1 mg/kg) for 7 days [13]. Compared with C57BL/6, the scopolamine-molded mice were proven to have impaired memory through the Morris Water Maze test [13]. After molding, the model mice and control were anesthetized with isoflurane, and the blood was collected from the right orbital vein. The blood samples were centrifuged at $3000 \times g$ at 4 °C for 10 min. Then, the supernatant plasmas were collected and stored at – 80 °C until analysis.

Exposure levels of alkaloids in different developmental and health conditions in human

All 550 individuals were given written informed consents before drawing blood. In addition, the participants and their relatives were informed that anonymized data might be used in clinical research

studies. Moreover, health examination and questionnaires were necessary. The Guardian was requested to do the questionnaires if participant could not answer. The survey involved various aspects, including name, age, nationality, gender, living habits, and contact information. Living habits included drinking and smoking. Detail information is shown in Table 1. Health examination about physiological states included neurodegeneration and any other disease. This experiment aimed to detect harmine, harmaline, harmane, and nor-harmane exposure levels in different developmental and physiological states. Furthermore, the difference of alkaloid exposure levels by gender, race, and lifestyle was detected. Blood sampling and numbering were directed by nurses in Kashi Prefecture First People’s Hospital and Shuguang Hospital affiliated to Shanghai University of Traditional Chinese Medicine. Serial numbers corresponded to detail information about every participant. The numbers were randomly allocated by sequence of arrival at hospital. Analysis about alkaloids in plasma was developed without further information except for serial numbers. Approximately 1 mL of venous blood was collected from participants who have fasted for at least 12 h. The blood samples were centrifuged at $3000 \times g$ at 4 °C for 10 min to obtain plasma. Then, plasma samples were stored at – 80 °C until analysis.

Table 1

Characteristics of healthy participants and AD patients

Characteristics of subjects	SH cohort	XJ cohort
Number	131	419
Gender (Women/men)	79/52	101/318
Age (mean ± SD)	28 ± 4	41 ± 12
Race (Han/Uyghur)	124/7	274/145
Drinking (Yes/No)	13/118	199/220
Smoking (Yes/No)	9/122	162/257
-: no relevant statistics.		

Statistical analysis

Statistical evaluation was performed with SPSS version 18.0 software, and the data were presented as mean ± SD. Unadjusted *P* values were reported as this study is an exploratory rather than a confirmatory analysis. This study aimed to confirm whether harmine, harmaline, harmane, and nor-harmane were endogenous and compare their exposure levels in different gender, nationality, and lifestyle. Thus, four separate stepwise logistic regression analyses were conducted, that is, one analysis each for harmine, harmaline, harmane, and nor-harmane as the dependent variable. Data distribution was evaluated graphically using histograms and Q–Q plots. Nonparametric and parametric tests were used in the study. T test was used to compare the concentrations of alkaloids among the different groups. A nonparametric test was applied as part of statistical analyses for non-normal distribution. Participants were divided into

two groups, and 60 years old was selected as the division point. Kruskal–Wallis H nonparametric test was used to compare the two groups. The threshold for statistical significance was set at $P < 0.05$ (2-tailed).

Abbreviations

βCBs: β-Carboline alkaloids;

5-HT: 5-Hydroxytryptamine;

5-HIAA: 5-Hydroxyindole-3-acetic acid;

ACh: Acetylcholine chloride;

AChE: Acetylcholinesterase;

AD: Alzheimer's disease;

Ch: Choline chloride;

L-Glu: L-Glutamic acid;

L-Phe: L-Phenylalanine;

L-Trp: L-Tryptophan;

L-Tyr: L-Tyrosine;

MAO-A: Monoamine oxidase A;

P–S reaction: Pictet–Spengler reaction;

Declarations

Acknowledgement

We would like to thank all volunteers who have participated in this study.

Funding

This work was supported by the National Natural Science Foundation of China (No.81872933, 81173119), the National Natural Science Foundation of China and Xinjiang Uygur Autonomous Region of China (No. U1130303), and the Technology Cooperation Projects of Science in Shanghai, China (No. 20015800100) awarded to Professor Chang-Hong Wang for financial support of this study.

Author Contribution Statement

Ning Cao performed most of the experiments and analyzed data and wrote the paper. Shuping Li detected the concentrations of harmine, harmaline and harmane in plasma of AD mode mice and control. Aimin Xu and Xiaoguang Zou collected plasma and basic information of volunteers. Zunji Ke provide idea of the experiment about pup rats. Gang Deng collected plasma of aging rats. Xuemei Cheng helped to develop the UPLC-MS/MS detection. Changhong Wang proposed the project and make revised and gave suggestions on the revision.

Availability of data and materials

All data generated or analyzed during this study are included in the published article. Some or all data generated or used during the study are available from the corresponding author by request.

Ethics approval and consent to participate

The study was approved by the institutional review committee of Kashi Prefecture First People's Hospital (NO.2017-03; Approval date: February 28, 2017) and was performed in accordance with the principles expressed in the Declaration of Helsinki. All study participants provided written informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Li S, Cheng XM, and Wang CH, A review on traditional uses, phytochemistry, pharmacology, pharmacokinetics and toxicology of the genus *Peganum*. *J Ethnopharmacol.* 2017; 203, 127-162.
2. Zhao T, Zheng SS, Zhang BF, Li YY, Bligh SW, Wang CH, et al, Metabolic pathways of the psychotropic-carboline alkaloids, harmaline and harmine, by liquid chromatography/mass spectrometry and NMR spectroscopy. *Food Chem.* 2012; 134, 1096-105.
3. Li S, Teng L, Liu W, Cheng X, Jiang B, Wang Z, et al, Pharmacokinetic study of harmane and its 10 metabolites in rat after intravenous and oral administration by UPLC-ESI-MS/MS. *Pharm Biol.* 2016; 54, 1-14.
4. Xie Z, Cao N, and Wang C, A review on the contents of β -carboline alkaloids in foodstuffs: a class of potential functional components or not? *Food Chemistry.* 2021; 348, 129067.

5. Louis ED, Factor-Litvak P, Liu X, Vonsattel JP, Galecki M, Jiang W, et al, Elevated brain harmaline (1-methyl-9H-pyrido[3,4-b]indole) in essential tremor cases vs. controls. *Neurotoxicology*. 2013; 38, 131-135.
6. Louis ED, Michalec M, Jiang W, Factor-Litvak P, and Zheng W, Elevated blood harmaline (1-methyl-9H-pyrido[3,4-b]indole) concentrations in Parkinson's disease. *Neurotoxicology*. 2014; 40, 52-56.
7. Louis ED, Zheng W, Applegate L, Shi L, and Factor-Litvak P, Blood harmaline concentrations and dietary protein consumption in essential tremor. *Neurology*. 2005; 65, 391-396.
8. Cao N and Wang C, Strictosidine synthase, an indispensable enzyme involved in the biosynthesis of terpenoid indole and β -carboline alkaloids. *Chinese Journal of Natural Medicines*. 2020;
9. Stockigt J, Antonchick AP, Wu F, and Waldmann H, The Pictet-Spengler reaction in nature and in organic chemistry. *Angew Chem Int Ed Engl*. 2011; 50, 8538-64.
10. Fabbri M, Delp G, Schmidt O, and Theopold U, Animal and plant members of a gene family with similarity to alkaloid-synthesizing enzymes. *Biochem Biophys Res Commun*. 2000; 271, 191-196.
11. Abu Ghazaleh H, Lallies MD, Nutt DJ, and Hudson AL, The modulatory action of harmaline on serotonergic neurotransmission in rat brain. *Brain Res*. 2015; 1597, 57-64.
12. Wang YX, Wang HX, Zhang LH, Zhang YP, Sheng YC, Deng G, et al, Subchronic toxicity and concomitant toxicokinetics of long-term oral administration of total alkaloid extracts from seeds of *Peganum harmala* Linn: A 28-day study in rats. *J Ethnopharmacol*. 2019; 238, 111866.
13. Li SP, Wang YW, Qi SL, Zhang YP, Deng G, Ding WZ, et al, Analogous beta-carboline alkaloids harmaline and harmine ameliorate scopolamine-induced cognition dysfunction by attenuating acetylcholinesterase activity, oxidative stress, and inflammation in mice. *Front Pharmacol*. 2018; 9, 1-16.
14. Association As, 2016 Alzheimer's disease facts and figures. *Alzheimers Dement*. 2016; 12, 459-509.
15. Kumar A, Singh A, and Ekavali, A review on Alzheimer's disease pathophysiology and its management: an update. *Pharmacol Rep*. 2015; 67, 195-203.
16. Hampel H, Mesulam MM, Cuello AC, Farlow MR, Giacobini E, Grossberg GT, et al, The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain*. 2018; 141, 1917-1933.
17. Perng CH, Chang YC, and Tzang RF, The treatment of cognitive dysfunction in dementia: a multiple treatments meta-analysis. *Psychopharmacology (Berl)*. 2018; 235, 1571-1580.
18. Tang KS, The cellular and molecular processes associated with scopolamine-induced memory deficit: A model of Alzheimer's biomarkers. *Life Sci*. 2019; 233, 1-6.
19. Mollon J, David AS, Zammit S, Lewis G, and Reichenberg A, Course of cognitive development from infancy to early adulthood in the psychosis spectrum. *JAMA Psychiatry*. 2018; 75, 270-279.
20. Guerreiro R and Bras J, The age factor in Alzheimer's disease. *Genome Med*. 2015; 7, 106.
21. Wang YX, Cao N, Guan HD, Cheng XM and Wang CH, Heme peroxidases is responsible for the dehydrogenation and oxidation metabolism of harmaline into harmine. *Chinese Journal of Natural Medicines*.(Under review)

22. Zhu H, Kercmar P, Wu F, Rajendran C, Sun L, Wang M, et al, Using strictosidine synthase to prepare novel alkaloids. *Current Medicinal Chemistry*. 2015; 22, 1880-1888.
23. Ma X, Panjikar S, Koepke J, Loris E, and Stockigt J, The structure of *Rauvolfia serpentina* strictosidine synthase is a novel six-bladed beta-propeller fold in plant proteins. *Plant Cell*. 2006; 18, 907-920.
24. Dakic V, Maciel RM, Drummond H, Nascimento JM, Trindade P, and Rehen SK, Harmine stimulates proliferation of human neural progenitors. *Peer J*. 2016; 4, 1-13.
25. Arib O, Rat P, Molimard R, Chait A, Faure P, and de Beaurepaire R, Electrophysiological characterization of harmine-induced activation of mesolimbic dopamine neurons. *Eur J Pharmacol*. 2010; 629, 47-52.
26. Jungner A, Vallius Kvist S, Romantsik O, Bruschettini M, Ekstrom C, Bendix I, et al, White matter brain development after exposure to circulating cell-free hemoglobin and hyperoxia in a rat pup model. *Dev Neurosci*. 2019; 41, 234-246.
27. Corriveau RA, Koroshetz WJ, Gladman JT, Jeon S, Babcock D, Bennett DA, et al, Alzheimer's Disease-related dementias summit 2016: national research priorities. *Neurology*. 2017; 89, 2381-2391.
28. Hargis KE and Blalock EM, Transcriptional signatures of brain aging and Alzheimer's disease: What are our rodent models telling us? *Behav Brain Res*. 2017; 322, 311-328.
29. Xia X, Jiang Q, McDermott J, and Han J-DJ, Aging and Alzheimer's disease: Comparison and associations from molecular to system level. *Aging Cell*. 2018; 17, 1-14.
30. Esquerda-Canals G, Montoliu-Gaya L, Guell-Bosch J, and Villegas S, Mouse models of Alzheimer's Disease. *J Alzheimers Dis*. 2017; 57, 1171-1183.
31. Swenson BL, Meyer CF, Bussian TJ, and Baker DJ, Senescence in aging and disorders of the central nervous system. *Translational Medicine of Aging*. 2019; 3, 17-25.
32. Bebia Z, Buch S, Wilson J, Frye R, Romkes M, Cecchetti A, et al, Bioequivalence revisited: Influence of age and sex on CYP enzymes. *Clinical Pharmacology & Therapeutics*. 2004; 76, 618-627.
33. Lopes GS, Bielinski SJ, Moyer AM, Black Iii JL, Jacobson DJ, Jiang R, et al, Sex differences in associations between CYP2D6 phenotypes and response to opioid analgesics. *Pharmgenomics Pers Med*. 2020; 13, 71-79.
34. Li S, Teng L, Liu W, Cheng X, Jiang B, Wang Z, et al, Interspecies metabolic diversity of harmaline and harmine in in vitro 11 mammalian liver microsomes. *Drug Test Anal*. 2017; 9, 754-768.
35. Bradford LD, CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics*. 2002; 3, 229-243.
36. Kim MJ, Byeon JY, Kim YH, Kim SH, Lee CM, Jung EH, et al, Effect of the CYP2D6*10 allele on the pharmacokinetics of clomiphen and its active metabolites. *Arch Pharm Res*. 2018; 41, 347-353.
37. Zuo LJ, Guo T, Xia DY, and Jia LH, Allele and genotype frequencies of CYP3A4, CYP2C19, and CYP2D6 in Han, Uighur, Hui, and Mongolian Chinese populations. *Genet Test Mol Biomarkers*. 2012; 16, 102-108.

38. Herraiz T, Guillen H, and Aran VJ, Oxidative metabolism of the bioactive and naturally occurring beta-carboline alkaloids, norharman and harman, by human cytochrome P450 enzymes. *Chem Res Toxicol.* 2008; 21, 2172-2180.
39. Huang D, Yu M, Yang S, Lou D, Zhou W, Zheng L, et al, Ethanol alters APP processing and aggravates Alzheimer-associated phenotypes. *Mol Neurobiol.* 2018; 55, 5006-5018.
40. Rehm J, Hasan OSM, Black SE, Shield KD, and Schwarzing M, Alcohol use and dementia: a systematic scoping review. *Alzheimers Res Ther.* 2019; 11, 1.
41. Zhao T, He YQ, Wang J, Ding KM, Wang CH, and Wang ZT, Inhibition of human cytochrome P450 enzymes 3A4 and 2D6 by beta-carboline alkaloids, harmine derivatives. *Phytother Res.* 2011; 25, 1671-1677.
42. Jiang B, Meng L, Zou N, Wang H, Li S, Huang L, et al, Mechanism-based pharmacokinetics-pharmacodynamics studies of harmine and harmaline on neurotransmitters regulatory effects in healthy rats: Challenge on monoamine oxidase and acetylcholinesterase inhibition. *J Phytomedicine* 2019; 62, 152967.
43. Yang Y, Cheng X, Wang C, Zheng L, Li Y, Li X, et al, Research on quality specification of the seeds of *Peganum harmala* L. of a uygur traditional medicine. *Chin Pharm J.* 2014; 49, 106-112.
44. He D, Wu H, Wei Y, Liu W, Huang F, Shi H, et al, Effects of harmine, an acetylcholinesterase inhibitor, on spatial learning and memory of APP/PS1 transgenic mice and scopolamine-induced memory impairment mice. *Eur J Pharmacol.* 2015; 768, 96-107.

Figures

Figure 1

Time schedule of the experimentation. A: The time schedule of the experimentation about the exposure levels of alkaloids in the developmental phase of newborn rats within 29 days of birth. B: The time schedule of the experimentation about the exposure levels of alkaloids and neurotransmitters in the growing process of 18-month-old rats (from youth to old stage).

Figure 2

Concentration of harmine in rat plasmas and various tissues in the developmental process of pup rats.

Figure 3

Concentrations of harmine in rat plasma with the growing process of 18-month-old rats (from youth to old stage).

Figure 4

Concentration of eight neurotransmitters in rat plasma with the growing process of 18-month-old rats (from youth to old stage). A: the concentration of 5-HIAA; B: the concentration of 5-HT; C: the concentration of ACh; D: the concentration of Ch; E: the concentration of Glu; F: the concentration of L-trp; G: the concentration of Glu; H: the concentration of Tyr.

Figure 5

Exposure regulation of different healthy states. A: wild-type mice compared with APP knock-in mice; B: scopolamine-mode mice compared with normal mice.

Figure 6

Results of human plasma determination. A: contents of harmine, harmane, and harmaline in plasmas of healthy individuals who were below 60 years old compared with those above 60 years old; B: concentrations of the abovementioned alkaloids in plasmas of healthy male individuals compared with female; C: concentrations of the abovementioned alkaloids in plasmas of the Han people compared with the Uighur people; D: concentrations of the abovementioned alkaloids in plasmas of healthy individuals who drink in daily life compared with those who were not; E: concentrations of the abovementioned alkaloids in plasmas of individuals who smoke in daily life compared with those who were not; Significant difference: * $P < 0.05$, *** $P < 0.001$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [AuthorChecklistFull.pdf](#)
- [Supplementarymaterials.docx](#)